ADENOVIRUS-MEDIATED TRANSFER OF HUMAN TGF-β1 ENCODING GENE TO THE RABBIT INTERVERTEBRAL DISC IN-VIVO UPREGULATES PROTEOGLYCAN SYNTHESIS

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INTRODUCTION: Disc degeneration and associated spinal disorders are a leading source of morbidity resulting in substantial pain and suffering and increased health care costs. Although the etiology and pathophysiology of intervertebral disc degeneration are largely unknown, few approaches are available clinically for the treatment or prevention of disc degeneration. Thompson and colleagues showed that addition of recombinant human TGF-β1 to canine disc tissue in culture could stimulate in-vitro proteoglycan synthesis, and they suggested that this growth factor might be used for the treatment of disc degeneration. Although recombinant growth factors have promising therapeutic properties, sustained delivery of the proteins to patients (especially for chronic conditions) would be difficult to accomplish with present technology. Adenovirus-mediated gene transfer of marker genes to the intervertebral disc in-vivo has been reported by Nishida and colleagues, resulting in long-term in-vivo transgene expression (up to 12 weeks). The objective of the current study was to determine the feasibility of direct, adenovirus-mediated transfer of a therapeutic gene to rabbit nucleus pulposus in-vivo. We hypothesized that direct adenovirus-mediated gene transfer can transfect the human TGF-β1 gene to the rabbit intervertebral disc cells in-vivo, resulting in increased TGF-β1 production and increased proteoglycan synthesis.

MATERIALS AND METHODS: Surgical procedures and in-vivo transduction: Five skeletally mature female New Zealand white rabbits, 4–5kg each, were used in this study. The anterior aspects of the L2-3, L3-4, and L4-5 intervertebral discs were exposed using a retroperitoneal approach. In three rabbits (n=9 disc), 20 µl of saline containing Ad/CMV-hTGF-β1 (6x10⁶ PFU) were injected through a 26-gauge hypodermic needle into the nucleus pulposus. In the fourth and fifth rabbits (n=3 discs each), 20 µl of saline with or without Ad/CMV-luciferase (6x10⁶ PFU) were injected, respectively. For each rabbit, the L1-2 intervertebral disc was used as the intact control. After one week, the animals were euthanized. Bioassays for determination of TGF-β1 production and proteoglycan synthesis: After sacrifice, the nucleus pulposus tissues were harvested and cultured in NeumanTytell serumless medium for 48 hours, after which the medium was extracted for ELISA to detect TGF-β1 production. New
medium containing $^{35}$S-sulfate (10 µCi/ml) was added to the cultures, and 48 hours later, the medium together with nucleus pulposus tissue was extracted and combined with an equal volume of solution containing 8M guanidine hydrochloride (GuHCl), 20 mM EDTA, and a mixture of proteinase inhibitors, and incubated at 60°C for 48 hours. For quantitative evaluation of the $^{35}$S-labeled proteoglycans, aliquots of the extracts were eluted on Sephadex G-25 in PD-10 columns, and the radioactivity of the newly synthesized proteoglycans was measured by a scintillation counter. All of the data were normalized by wet tissue weight, and statistical analysis performed using one-way ANOVA with Fisher's Protected LSD post-hoc test. Two discs from the Ad/CMV-hTGFβ1 group were excluded from the data analysis because of technical problems during surgery.

RESULTS: In-vivo injection of Ad/CMV-TGFβ1 into the nucleus pulposus resulted in a significant increase in the production of active TGF-β1 over that of control discs (p<0.05). The average proteoglycan synthesis in the discs that received an Ad/CMV- TGF-β1 injection was over six times greater than that of the intact control discs (p<0.05).

DISCUSSION: Disc degeneration is a type of aging process, and this process is inevitable. Although the mechanisms are not well known, some cases of disc degeneration progress faster than the aging process: for example, secondary degeneration in discs adjacent to long fusion or in discs following annulus injury. The current study has demonstrated that human TGF-β1 encoding genes can be transferred to rabbit nucleus pulposus cells in-vivo, and that transfer of the genes induces increased production of active TGF-β1 - even without any artificial activation procedures such as acidification of latent TGF-β1. Further, we observed significantly increased proteoglycan synthesis, suggesting that this method might be used for the treatment of intervertebral disc degeneration to maintain or increase matrix synthesis. That we can genetically modify intervertebral disc cells and hence influence disc internal biological environment raises the possibility that gene transfer might also enhance disc mechanical properties, and possibly delay the onset of secondary disc degeneration in situations where the disc is exposed to severe mechanical environments. One potential clinical application therefore would be to inject the adenovirus construct encoding for TGF-β1 production into the adjacent disc at the time of fusion surgery.

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